Supplementary figures

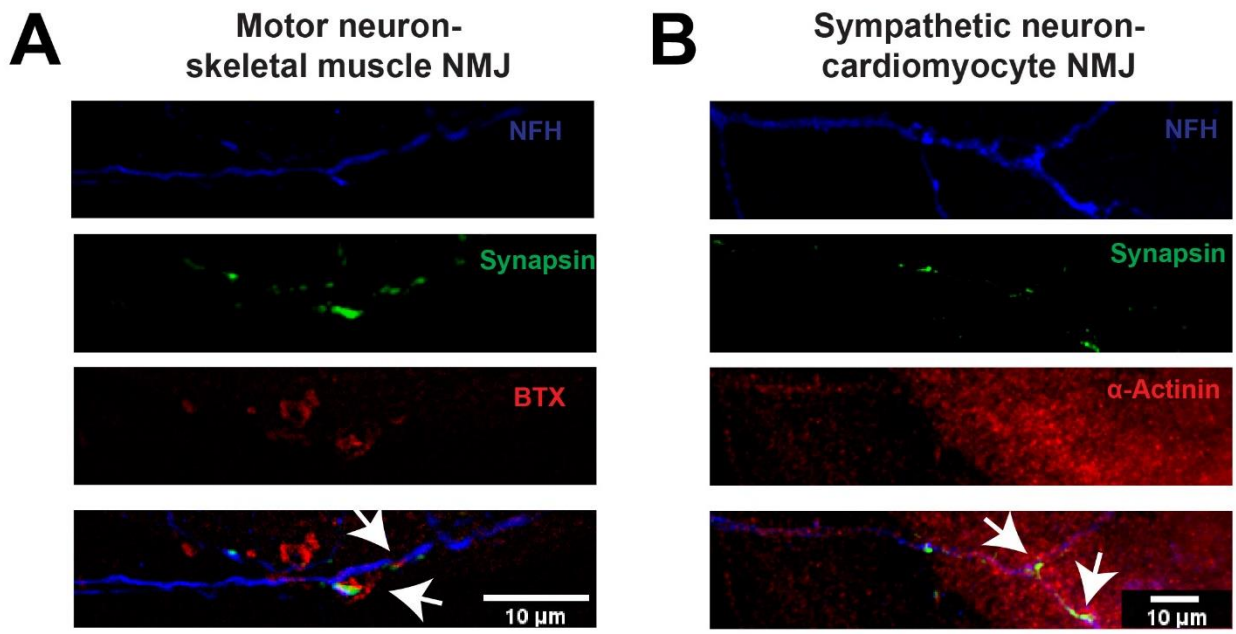


Fig. S1. MN and SN form synapses in an *in vitro* co-culture system. (A-B) Confocal images displaying co-localization of neuronal marker (NFH-blue), pre-synaptic marker (Synapsin I-green), and post-synaptic marker (BTX in skeletal muscles and α-actinin for cardiomyocytes) in MN-skeletal muscle co-culture (A) and SN-cardiomyocyte co-culture (B). Scale bar = 10µm.

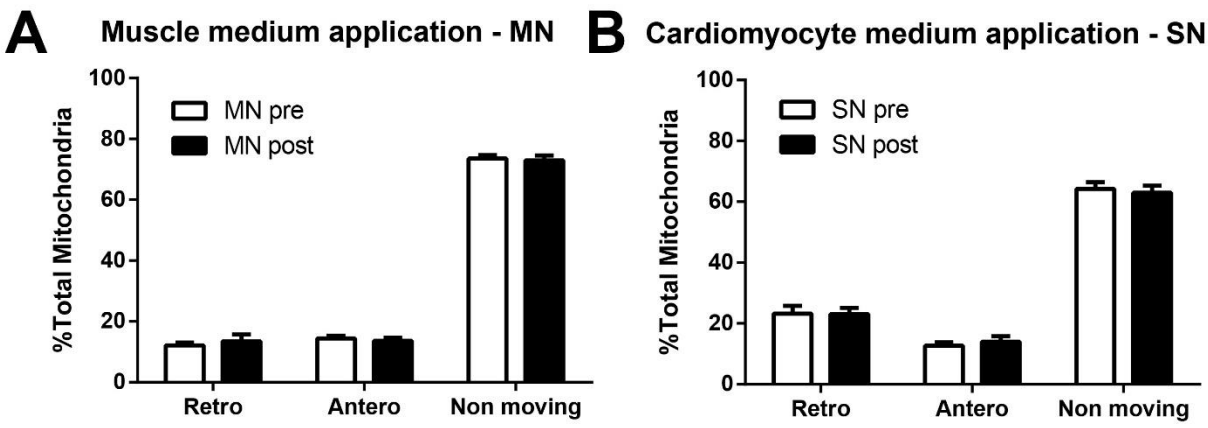


Fig. S2. Muscle conditioned media does not influence mitochondrial axonal transport. (A-B) Mitochondrial axonal transport analysis of MN (A) or SN (B) explants grown in MFC, before and after application of muscle (A) or cardiomyocyte (B) conditioned media for 3 hours. There was no apparent change in overall transport or directionality. n=8 MFCs for MNs and n=7 MFCs for SNs, at least 8 axons per MFC. The same chambers were imaged in the pre and post condition media application conditions. Two-way Anova with Holm-Sidak post-hoc test. Each graph contains at least 3 independent biological repeats. Data error bars represent mean ± SEM.

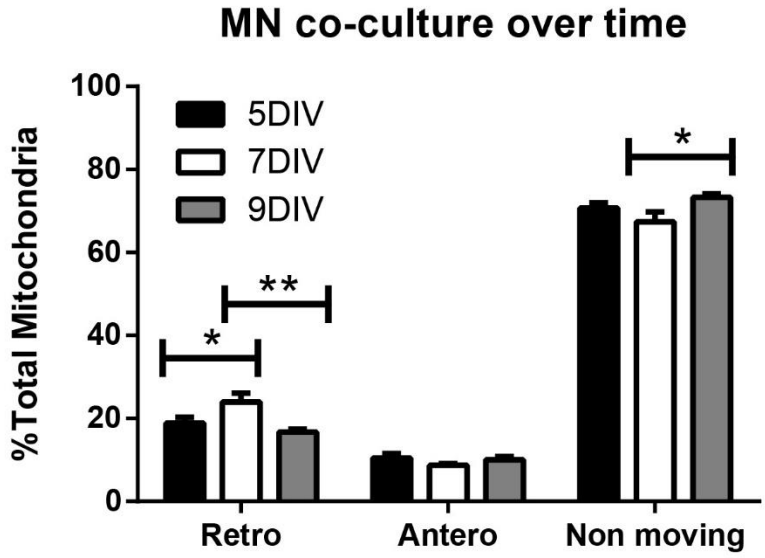
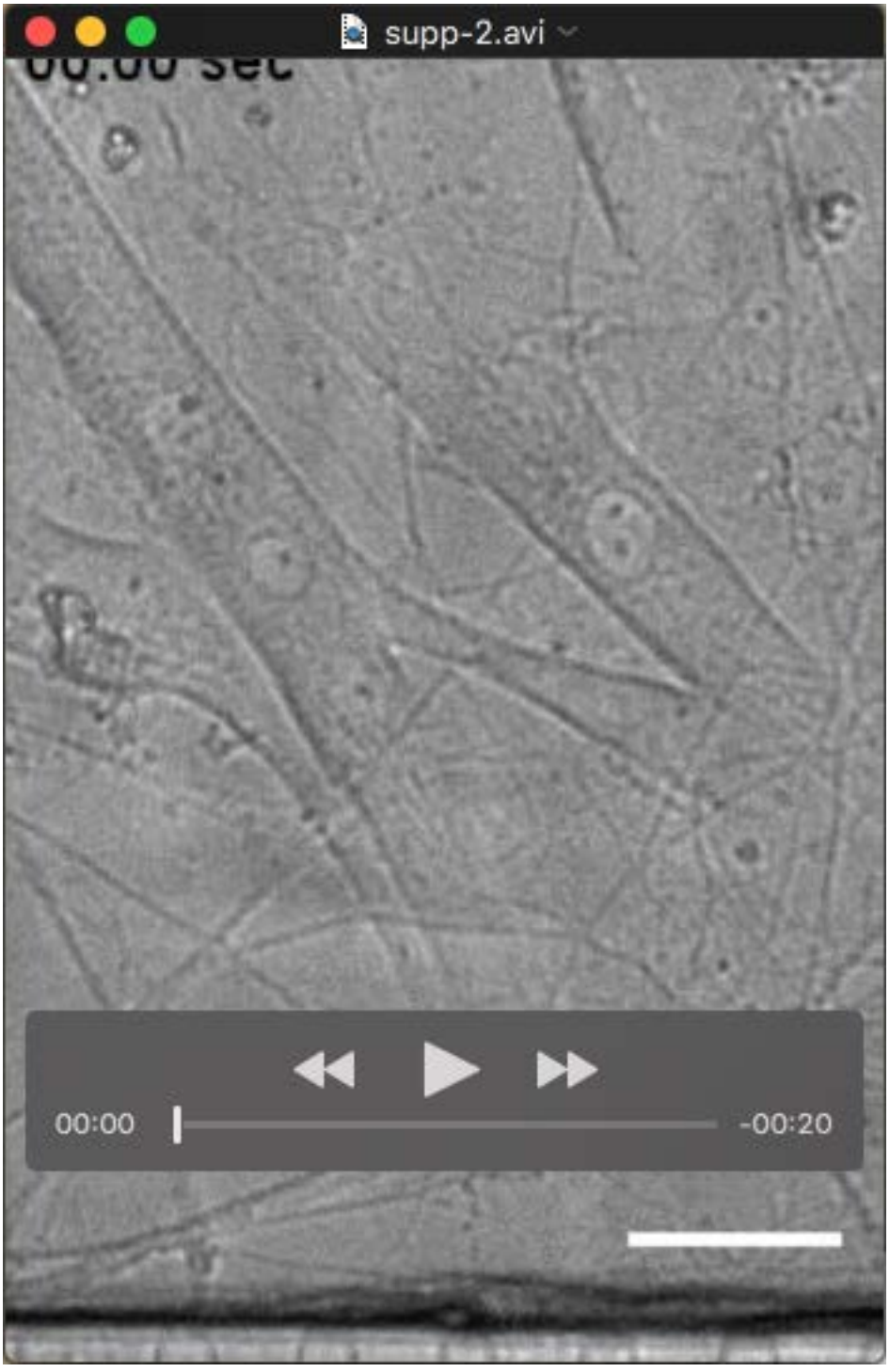
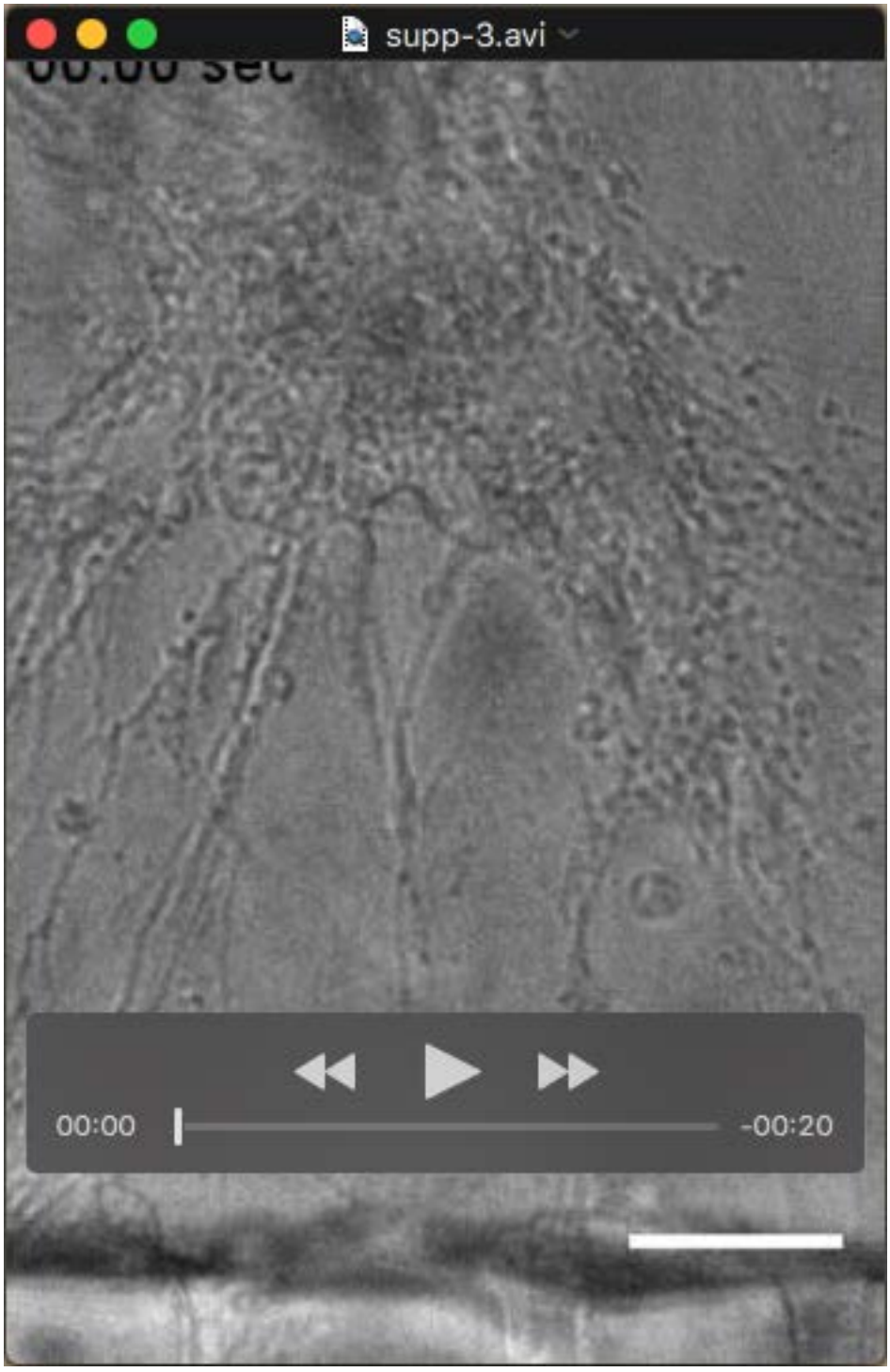


Fig. S3. MN mitochondrial axonal transport is only slightly influenced by NMJ formation. (A-B) Mitochondrial axonal transport analysis of MN explants in co-culture with skeletal muscles at time points 5,7 and 9 DIV. A slight increase in retrograde transport was observed at 7 DIV compared to 5 DIV but this was reversed at 9 DIV. **p <0.01, *p<0.05, two-way Anova with Holm-Sidak post-hoc test. n=8 MFCs per condition, at least 8 axons per MFC. The same chambers were imaged over time.

Each graph contains at least 3 independent biological repeats. Data error bars represent mean ± SEM.



Movie 1. Skeletal muscle contraction in the presence of overlaying MN. 20X magnification of 1000 frames; 30-second movie of muscles innervated by MN in MFC. Scale bar=20μm.



Movie 2. Cardiomyocyte contraction in the presence of overlaying SN. 20X magnification of 1000 frames; 30-second movie of cardiomyocytes innervated by SN in MFC. Scale bar=20μm.